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RELATIVE INSECTICIDAL VALUE OF COMMERCIAL GRADES OF PYRETHRUM

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RESULTS OF PREVIOUS EXPERIMENTS

It has been the generally accepted opinion, particularly in the trade, that the effectiveness of insect powder (pyrethrum) varies greatly, depending on the maturity of the flowers[2] from which it was made. Powder made from flowers known in the trade as "closed" has been considered the most effective, that from "open" flowers the least effective, and that from "half-closed" or mixed flowers of intermediate effectiveness. The closed flowers, and the powder made from them, therefore, have always commanded the highest price. This opinion does not seem to have been based on any experimental data, and how it originated is not clear. McDonnell, Roark, and Keenan, in their work on *Chrysanthemum cinerariaefolium* (*5*),[3] showed that the greatest activity (against roaches) is in the fruit (achenes).[4] As much of the fruit from very mature flowers may be lost during curing and handling, the effectiveness of powder made from such flowers would be materially reduced, a fact which may account for the observation that commercial open flowers may be low in effectiveness.

Investigations by several European workers at about the same time, reported by Juillet, d'Everlange, and Ancelin (*3*), showed that the generally accepted view on the distinction between the effectiveness of powders of various commercial grades does not merit the credence accorded it.

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[1] The writers are indebted to L. J. Bottimer, Wallace Colman, and G. D. Reynolds for assistance in conducting the field tests reported in this bulletin.

[2] The word "flower" as used in this bulletin refers to the flower head composed of disk and ray florets.

[3] Italic numbers in parentheses refer to "Literature cited," p. 9.

[4] Achene, botanically, is a small dry, indehiscent, 1-seeded carpel with a leathery pericarp, and popularly known as the seed.

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Fryer, Tattersfield, and Gimingham (*1, p. 445*), in a report on the results of their work with alcoholic extracts of pyrethrum flowers grown in England from seed obtained from various sources, state that "the toxicities of extracts of equal weights of pyrethrum flowers tested at different stages of development differed very little." These investigators point out further that harvesting the crop in the closed stage causes a loss in actual yield of flowers per unit area of nearly 60 per cent, as compared with the yield when the crop is taken with the flowers fully open.

It has also been frequently stated that powder made from flowers grown in Japan is less effective than that from flowers from Dalmatia.

TESTS OF POWDERS AGAINST INSECTS

To obtain more definite information on these questions, pyrethrum flowers grown in Europe, representing the three commercial grades (closed, half-closed, and open), and flowers from Japan, bought on the open market, were ground into powder and tested against aphids. All of the flowers used were *Chrysanthemum cinerariaefolium*. The tests were started in 1926 and were conducted at intervals over a period of two years.

MATERIALS TESTED

The following commercial grades of flowers were used: Japanese flowers (samples 4 and 14); closed Dalmatian flowers (samples 5, 15, and 16); open Dalmatian flowers (samples 6, 10, 13, and 20); and half-open Dalmatian flowers (sample 7). All the samples were of the crop of 1926, except samples 5 and 7, which were of the crop of 1925, and samples 6 and 20, the crop year of which could not be determined.

Each lot of flowers was examined microscopically. Representative samples of Dalmatian flowers, consisting of about 50 grams from each bale, were separated into their different flower components. (Table 1.)

No attempt was made to separate the Japanese flowers into their principal parts, as they had been baled under hydraulic pressure and the various components were so broken that accurate separation was impossible.

TABLE 1.—*Microscopical separation of pyrethrum flowers*

Sample No.	Grade	Achenes	Disk florets	Ray florets	Receptacles	Miscellaneous [1]
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
5	Closed Dalmatian	13.0	34.0	24.8	24.0	4.2
15	do	6.9	44.4	19.9	28.0	.8
16	do	6.5	43.2	21.0	28.0	1.3
6	Open Dalmatian	57.1	5.5	13.0	18.0	6.4
10	do	47.9	18.8	16.0	16.4	.9
20	do	51.4	14.0	14.8	18.3	1.5
13	do	47.4	13.4	19.8	18.3	1.1
7	Half-open Dalmatian	34.7	23.1	19.9	22.0	.3

[1] Small pieces of stems, bracts, etc.

As would be expected, the closed flowers have a higher proportion of disk and ray florets than the other grades, and the open flowers have a very much higher porportion of achenes (fruit), the half-open flowers occupying an intermediate position.

The flowers were powdered by running them two or three times through a mill of the impact type, when all but 5 to 7 per cent would pass an 80-mesh sieve. That portion remaining on the sieve was further pulverized in a ball mill to pass a 70-mesh sieve.

CHEMICAL COMPOSITION

Chemical analyses of the powders were made. The results are given in Table 2.

TABLE 2.—*Chemical examination of pyrethrum flowers*

Sample No.	Grade	Moisture	Total ash	Acid-insoluble ash	Ether extract	Nitrogen
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
5	Closed Dalmatian	6.32	7.20	0.16	6.12	1.58
15	do	7.67	6.87	.15	6.05	1.76
16	do	6.43	6.54	.12	7.03	1.92
6	Open Dalmatian	6.38	7.09	.18	6.94	1.46
10	do	5.97	7.09	.26	6.98	1.40
20	do	6.40	6.63	.14	7.07	1.45
13	do	6.70	6.70	.13	7.08	1.64
7	Half-open Dalmatian	6.61	7.93	.18	6.30	1.56
4	Japanese	6.25	7.49	.33	6.94	1.69
14	do	6.39	7.17	.22	6.35	1.79

The chemical results show no striking differences between the flowers of the various types. On the basis of the averages for the different grades, the ash is a little higher in the Japanese than in the other grades, and the ether extract is slightly higher and the nitrogen the lowest in the open flowers.

ACTIVE CONSTITUENTS

The determination of the active constituent of insect flowers baffled the skill of the most careful investigators for many years. Finally, two Swiss chemists, Staudinger and Ruzicka (*7*), isolated and determined the chemical structure of two toxic constituents, to which they gave the names Pyrethrin I and Pyrethrin II, and published methods for their quantitative determination. They state that these constituents are present in the flowers to the extent of only from 0.2 to 0.3 per cent and that they consist of approximately 40 per cent of Pyrethrin I and 60 per cent of Pyrethrin II. No results showing the distribution of these compounds in the various flower parts are reported, however. In a later article, Staudinger and Harder (*6*) state that the content of the active principles may in favorable cases be 0.6 per cent. They state also that no great difference was found between open, half-open, and closed flowers.

Tattersfield, Hobson, and Gimingham (*8, p. 296*) report results on flowers obtained from different sources ranging from 0.6 to 1.2 per cent total pyrethrins, made up of approximately equal quantities of Pyrethrin I and Pyrethrin II (determined by the acid method). Tested in alcoholic solution against *Aphis rumicis* Linné, Pyrethrin I was found to be "about ten times as toxic to these insects as Pyrethrin II." The data available were insufficient "to show a significant correlation between the size of flower heads and the content of poison."

Gnadinger and Corl (*2*) developed a method for the determination of the active constituents of pyrethrum based on their action in reducing alkaline copper solution, similar to the action of the reducing sugars. The method does not differentiate between Pyrethrin I and Pyrethrin II. These investigators found total pyrethrins from 0.40 to 1.21 per cent in the samples of pyrethrum flowers and powders which they tested by this method. The pure pyrethrins were found to be extremely toxic to cockroaches, Pyrethrin I being slightly more toxic than Pyrethrin II.

The active constituents in the powders used in the tests here described were determined by three methods: (1) The acid method of Staudinger and Harder (*6*), (2) the modification of this method by Tattersfield, Hobson, and Gimingham (*8*), and (3) the method of Gnadinger and Corl (*2*).

TABLE 3.—*Active principles in pyrethrum powders determined by three different methods*

Sample No.	Grade	Method of Staudinger and Harder			Method of Tattersfield, Hobson, and Gimingham			Method of Gnadinger and Corl [1]
		Pyrethrin I	Pyrethrin II	Total	Pyrethrin I	Pyrethrin II	Total	Pyrethrins I and II
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
5	Closed Dalmatian	0.41	0.44	0.85	0.11	0.44	0.55	0.40
15	do	.47	.42	.89	.13	.42	.55	.41
16	do	.36	.41	.77	.07	.41	.48	.39
	Average	.41	.42	.84	.10	.42	.53	.40
6	Open Dalmatian	.54	.53	1.07	.19	.53	.72	.59
10	do	.42	.56	.98	.09	.56	.65	.39
13	do	.38	.33	.71	.08	.33	.41	.43
20	do	.24	.48	.72	.05	.48	.53	.40
	Average	.40	.48	.87	.10	.48	.58	.45
7	Half-open Dalmatian	.36	.46	.82	.11	.46	.57	.38
4	Japanese				.08	.59	.67	.71
14	do	.68	.80	1.48	.18	.80	.98	.62

[1] The determinations reported by this method are by Gnadinger and Corl and it is through their courtesy that these results are published.

From the variations shown on the same samples by the different methods it is evident that the methods are not all that might be desired. The averages of the results by the different methods are in the same order for the different grades of flowers however—the closed Dalmatian flowers being the lowest in total pyrethrins, the open Dalmatian flowers very slightly higher, and the Japanese flowers the highest, although the number of samples involved is too small to serve as a basis for drawing definite conclusions. As great differences are shown between samples of the same grade as between those of different grades.

TESTS OF EFFECTIVENESS

As the undiluted powders used as dusts under the conditions that prevailed in these tests would have given practically a 100 per cent mortality, it was necessary to run a number of preliminary experiments with mixtures containing various percentages of the powdered

flowers in order to obtain a mixture that would give a kill of between 25 and 75 per cent. Figures in this range are much more reliable statistically than those of either extreme. Mixtures of 20 per cent pyrethrum and 80 per cent wheat flour (by weight) generally fell within this range, and preparations made by this formula were used in all of the tests here considered.

For each test, 15 small potted cabbage plants infested with aphids (*Myzus persicae* Sulz.) were used. The number of aphids per plant varied from 75 to 300. The aphids on each plant were counted, and the individual plants were then carefully and thoroughly dusted and placed in the greenhouse. A paper collar was fixed around the stem of each plant to catch the aphids that dropped off, and an untreated leaf was placed at the base of the stem on which any aphids that were knocked off but not killed could take refuge. Although observations indicated that practically all of the aphids affected fell from the plants within an hour, the final observations were not made until about 24 hours after treatment. The number of living aphids on the plant and on the leaf was then counted, and the percentage of dead on each plant was calculated.

Most of the aphids affected fell from the plants within an hour after the application. Many of these did not move again, except to flex their appendages, but others crawled about in an apparently dazed condition. A few, seemingly unhurt, were able to settle and feed, often finding their way to the leaf at the base of the plant. The aphids included under the term "dead" were those that dropped off the plants and failed to settle on the leaves at the base of the cabbage plants.

At least five experiments, covering not less than 75 plants, were made with each material. The mean dead and its probable error were computed on the basis of the percentage of dead from each plant considered as a unit, and of the total number of aphids on all the plants. For computing the probable error of the mean, the formula $0.6745\sqrt{\frac{\Sigma v^2}{n(n-1)}}$ was used. This was taken as a measure of the efficiency of the sample in question.

EXTERNAL FACTORS

Study of a representative series of these tests showed that the ratio of effectiveness between two mixtures remained nearly constant in parallel tests. It was soon noted, however, that the efficiency of a given sample varied greatly from day to day, owing probably to the effect of external factors, such as light, humidity, and temperature. As it was impossible to control these factors, it was necessary to test on the same day and at the same time the mixtures that were to be directly compared. In this way the effect of the external factors would be the same on each sample and the results would be comparable, the pyrethrum dust used being the only variable factor.

In order to obtain some measure of the effect of humidity and temperature, 43 tests were selected and correlation coefficients computed. The correlation coefficient for the percentage dead and humidity was 0.56 ± 0.07. That for the percentage dead and temperature was 0.23 ± 0.10. These correlation coefficients indicate that the relative humidity is much more important than the temperature.

Under the conditions that prevailed it was not feasible to measure and accurately evaluate the effect of light, but the indications are that this factor is at least as important as the humidity. This phase of the problem is being investigated.

FLOWERS OF DIFFERENT GRADES

One sample of half-closed Dalmatian flowers, two samples of open Dalmatian flowers, three samples of closed Dalmatian flowers, and two samples of Japanese flowers were tested, 75 plants being used in each test. The results are shown in Table 4.

TABLE 4.—*Effectiveness of pyrethrum of different commercial grades against Myzus persicae*

Experiment No.	Sample No. and grade of pyrethrum	Aphids	Dead (mean)	Difference / error
		Number	*Per cent*	
1	No. 4, Japanese	5,584	70.1±1.8	
	No. 14, Japanese	5,424	55.6±2.0	
			14.5±2.7	5.4
2	No. 6, open Dalmatian	5,888	69.4±1.2	
	No. 5, closed Dalmatian	5,796	58.0±1.4	
			11.4±1.8	6.3
3	No. 6, open Dalmatian	5,888	69.4±1.2	
	No. 10, open Dalmatian	5,921	44.6±1.4	
			24.8±1.8	13.8
4	No. 16, closed Dalmatian	6,371	64.5±1.2	
	No. 5, closed Dalmatian	5,796	58.0±1.4	
			6.5±1.8	3.6
5	No. 6, open Dalmatian	5,888	69.4±1.2	
	No. 16, closed Dalmatian	6,371	64.5±1.2	
			4.9±1.7	2.9
6	No. 16, closed Dalmatian	6,371	64.5±1.2	
	No. 10, open Dalmatian	5,921	44.6±1.4	
			19.9±1.8	11.1
7	No. 5, closed Dalmatian	5,796	58.0±1.4	
	No. 10, open Dalmatian	5,921	44.8±1.4	
			13.2±2.0	6.6
8	No. 15, closed Dalmatian	6,004	59.1±1.8	
	No. 7, half-closed Dalmatian	5,347	50.5±2.0	
			8.6±2.7	3.2
9	No. 4, Japanese	5,584	70.1±1.8	
	No. 15, closed Dalmatian	6,004	59.1±1.8	
			11.0±2.5	4.4
10	No. 14, Japanese	5,425	55.6±2.0	
	No. 7, half-closed Dalmatian	5,347	50.5±2.0	
			5.1±2.8	1.8
11	No. 4, Japanese	5,584	70.1±1.8	
	No. 7, half-closed Dalmatian	5,347	50.5±2.0	
			19.6±2.7	7.3
12	No. 15, closed Dalmatian	6,004	59.1±1.8	
	No. 14, Japanese	5,425	55.6±2.0	
			3.5±2.7	1.3

A significant difference in efficiency was found in experiments 1, 2, 3, 6, 7, 9, and 11. The results in experiments 10 and 12 do not show

a significant difference. In experiments 4, 5, and 8 the differences are probably significant, although the ratio of difference to error is not great enough to establish this with certainty.

The greatest difference was 24.8 per cent, between two samples of open flowers (experiment 3). In two cases (experiments 2 and 5) open flowers were superior to closed flowers, but this is reversed in experiments 6 and 7, in which samples of closed flowers were the more effective. The open flowers used in experiments 6 and 7 were the least effective of any of the samples tested. There was a significant difference between two samples of the same commercial grade in experiments 1 and 3, and probably in experiment 4, and in four cases this was greater than the difference between two samples of different grades.

These results show that the commercial grading does not furnish an accurate criterion of the effectiveness of the pyrethrum and that individual samples in one grade may vary more widely than samples from different grades.

FLOWER PARTS

An attempt was made to ascertain which portion of the pyrethrum flower contains the greatest amount of the insecticidal principle. For this purpose a part of sample 13, open Dalmatian flowers, was separated into nearly pure samples of achenes, disk florets, and receptacles. (The quantity of ray florets was too small for the tests.) These were ground, sifted, and tested in the same manner as the other samples. The chemical analyses of these powders are given in Table 5, and the results of tests on aphids in Table 6. One hundred and five plants were used in each test.

Table 5.—*Chemical examination of flower parts*[1] *of sample 13, open Dalmatian flowers*

Part of flower	Moisture	Total ash	Acid-insoluble ash	Ether extract	Nitrogen
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Achenes	6.11	5.07	0.17	6.65	1.51
Disk florets	8.41	6.81	.19	5.45	1.49
Receptacles	7.87	7.37	.26	3.65	1.18

[1] Not enough material was available for the determination of the pyrethrins.

Table 6.—*Results of tests against Myzus persicae with the achenes, disk florets, and receptacles from pyrethrum flower heads, sample 13, open Dalmatian flowers*

Part of flower	Aphids	Dead (mean)	Difference / error
	Number	*Per cent*	
Achenes	8,714	67.4±1.1	
Disk florets	8,902	50.3±1.3	
		17.1±1.7	10.1
Achenes	8,714	67.4±1.1	
Receptacles	8,250	29.3±1.3	
		38.1±1.7	22.4
Disk florets	8,902	50.3±1.3	
Receptacles	8,250	29.3±1.3	
		21.0±1.8	11.7

The results in Table 6 show that the seeds are the most effective, the disk florets next, and the receptacles the least effective and that the differences are in every case significant. These results agree with those previously reported (*5*), which showed that the relative effectiveness against roaches (time required to kill) of the flower parts, beginning with the most efficient, are as follows: Fruit (achenes) disk florets, receptacles, ray florets, and involucre.

As the achenes are the most effective portion of the flower, it would seem that the more mature flower would have the greatest insecticidal value, although this is contrary to the general opinion of the pyrethrum trade, which considers the closed flower superior to the open flower. An explanation of this may lie in the fact that in the open or mature flower the achenes are shed rather readily and may often be lost during curing or sift out of the bales, so that the material when ground consists largely of receptacles, which the tests show are much less effective. Examination of commercial samples of open flower collected at the ports of entry frequently shows that a large proportion of the achenes has been lost.

The foregoing tests were made with 20 per cent pyrethrum and should not be considered as indicating the actual effectiveness of these pyrethrums, as all of them no doubt would be effective against the insects ordinarily controlled by pyrethrum if used undiluted.

CONCLUSIONS

On the basis of the experiments and tests here reported, neither the commercial grade of pyrethrum flowers nor the locality in which the plants were grown can be accepted as giving an accurate criterion of the effectiveness of the product against insects. These experiments also show that there may be a greater difference in efficiency between two samples of the same commercial grade than between two samples of different commercial grades. This difference in effectiveness may be due to, or influenced by, one or more of the following factors: (1) Pyrethrums of different varieties, or grown under different climatic and soil conditions, may contain different amounts of the active constituents (*1*), (*4*), and (2) conditions existing at the time of harvesting and the method of curing the flowers as well as the conditions encountered in shipping and storing them probably have an influence on their effectiveness. It is impossible under commercial conditions to harvest the product when all flowers are in exactly the the same stage of growth. Furthermore, open (mature) flowers are likely to have lost a certain proportion of the achenes, which are the most effective portion of the flowers.

Tests with the powdered achenes showed them to be significantly more effective than the disk florets and the disk florets more effective than the receptacles. In view of this, and the further fact that the greatest yield is secured when the achenes have reached maturity, it would appear that the most economical time to harvest the flowers would be when fully ripened, provided the crop can be handled so as to avoid loss of the achenes.

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